THE EFFECT OF EGG YOLK IN DIETS ON ANAPHYLACTIC ARTHRITIS (PASSIVE ARTHUS PHENOMENON) IN THE GUINEA PIG*

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That there may be a relation between diet and the degree of susceptibility to anaphylactic reactions is well recognized. To investigate this possibility the guinea pig has been selected as an appropriate experimental animal. Fischel, Benacerraf, and Kabat showed that in the guinea pig there could be produced a passive Arthus reaction in which the response is quantitatively related to the amount of antibody used (1). Ungar and his colleagues found that the mean joint swelling measurement gives a reliable indication of the degree of inflammation associated with the Arthus anaphylactic arthritis in the guinea pig (2). Coburn and Haninger reported that, associated with the tissue injury of anaphylactic arthritis, there is a rise in the serum level of some substance which reacts with diphenylamine (DPA reaction) to give a purple color (3). Both the joint swelling accompanying the Arthus phenomenon and the increased degree of the DPA reaction following the passive anaphylactic arthritis have proved to be expressions of the intensity of this inflammatory response (4). The purpose of this report is to present experimental evidence that whole egg yolk and certain fractions of egg yolk, when used as supplements to the normal diet, inhibit anaphylactic arthritis in the guinea pig, as measured by joint swelling and the DPA reaction.

Methods

1. Technique for Determining Serum DPA Values:

The semimicro procedure used was described originally by Ayala, Moore, and Hess (5) and applied by Coburn and Haninger (3). Each observation is the corrected optical density of the purple color determination. Results represent the differences in optical densities between two samples of serum, one obtained about 24 hours prior to the challenge, the other obtained 20 hours after the peak of the swelling reaction.

2. Anaphylactic Arthritis:

Except for Experiment 6 each Arthus reaction was limited to a single ankle joint. The technique used throughout was that described by Ungar *et al.* (2).

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3. Fraction of Egg Yolk Powder:[1]

(*a*) *Isolation of Acetone-Soluble, Alcohol-Soluble, and Protein Fractions.*—Three hundred pounds of dry egg yolk powder was extracted exhaustively with acetone at room temperature. Four acetone extractions were made.

The acetone-insoluble residue was dried *in vacuo* at 120°F. The residue was then exhaustively extracted with ethyl alcohol at 70°F. in a similar manner as employed for the acetone extraction. The alcohol-soluble fraction was distilled in vacuum to remove the solvent. Water was added at the end in order to remove all the alcohol. The alcohol soluble material amounted to 61 pounds of 5.2 per cent solids.

The insoluble protein residue was dried in vacuum at 120°F.

(*b*) *Partition of Alcohol-Soluble Material in Skellysolve B and 85 per cent Ethanol.*—Dry alcohol-soluble material, 250 gm., was dissolved in Skellysolve B and extracted with 85 per cent alcohol. The extraction was continued until the alcoholic extracts contained 224 gm. and the Skellysolve B-soluble material amounted to 31 gm.

(*c*) *Fractionation of Alcohol-Soluble Material.*—Seventeen pounds of alcohol-soluble material was stirred with 7 gallons of warm absolute alcohol for 2 hours. The mixture was chilled in a cold room overnight and the supernatant alcoholic solution decanted. This process was repeated twice more. Alcohol was removed from these fractions under vacuum. We thus obtained a cold alcohol-soluble fraction (crude lecithin) which contained most of the lecithin and a cold alcohol-insoluble fraction (crude sphingomyelin) which consisted of cephalin, sphingomyelin, and impurities.

RESULTS

Preliminary Experiment.—The first experiment was a comparison of pigs bred on normal and experimental diets:

(*a*) 15 females on stock of pellets[2] with carrots and lettuce; (*b*) 15 females on identical diet in which the pellets were supplemented with 10 per cent whole egg yolk powder by weight. Water was made available. No measurements were kept of the wastage of pellets. When the offspring were born they were maintained on these two diets until they reached about 300 gm. in weight. At that time 12 control pigs and 9 pigs born and raised on the experimental diet were given a passive Arthus arthritis. It was found that the results were the same with two antigen-antibody systems, either an egg albumin–anti-egg albumin system or with a horse serum–anti-horse serum system. The mean swelling index (M.S.I.) for the pigs on the experimental diet, induced by the antigen-antibody reaction, was approximately 50 per cent of that of pigs on the regular diet.[3]

Experiment 2.—The second experiment was done with three fractions of whole egg yolk.

In this case 5 females were bred on the normal diet and 15 on each of the three experimental diets. Pellets were made with three egg yolk fractions: (*a*) fat-free material (protein fraction, etc.); (*b*) alcohol-soluble (phospholipids, etc.); (*c*) acetone-soluble (cholesterol, etc.). The pellets for each experimental diet contained one fraction, in an amount equivalent to 10 per cent by weight of the whole egg yolk. The pigs on diets (*a*) and (*b*) bred readily as did the

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[1] Prepared at the Wilson Laboratories.

[2] Rockland Guinea Pig Diet, Arcady Farms Milling Company, Chicago.

[3] Determinations made by Dr. Georges Ungar and Miss Evelyn Damgaard.

controls. However, there were many miscarriages among pigs receiving the acetone-soluble fraction. The progeny of all three groups gained weight and grew about the same as the controls. The offspring were all challenged with the passive Arthus arthritis when they reached a weight of about 300 to 350 gm.

The results indicated that the observations made on whole egg yolk could be repeated with the alcohol-soluble material. However, most of the pigs which had received the acetone-soluble material died as soon as the antibody was injected, prior to the administration of antigen. The appearance of the lungs was similar to that seen in anaphylaxis. The observations on pigs given the protein and alcohol-soluble fraction are shown in Table I.

TABLE I

Summary of Joint Swelling in Arthus Arthritis of the Guinea Pig

Diet	No. guinea pigs	M.S.I.	±S.E.	*P*	Inhibition
					per cent
Control	5	5.3	0.38	—	—
Protein residue	11	4.9	0.23	0.4	—
Alcohol-soluble fraction	21	2.8	0.28	<.001	47.1

The combined findings indicate that animals fed with alcohol-soluble fraction received the same protection as those fed with a supplement of whole egg yolk. The protein fraction appeared inactive. Of the guinea pigs fed on the acetone fraction, only 4 survived the antibody-antigen reaction; the joint swelling was approximately the same as that of the controls.

At autopsy the organs of the pigs which were born and bred on diets containing the protein or the alcohol-soluble fractions appeared normal. In contrast, the pigs which had received the acetone-soluble material, from which all acetone had been removed, had enormous, fatty livers. Microscopic sections showed a typical fatty cirrhosis throughout the liver in each instance.

Determinations were made for free and total cholesterol of pooled guinea pigs sera from the controls and the three groups on the experimental diet. Male and female pigs were used in each pool. The results are given in Table II.

TABLE II

Blood Cholesterol Levels of Guinea Pigs on Normal and Supplemented Diets

	Total	Free
	mg. per cent	*mg. per cent*
Normal	40	*
Protein residue	32	*
Alcohol-soluble fraction	78	18
Acetone-soluble fraction	553	88

* Too small to be determined accurately.

Experiment 3.—The third experiment was done with two fractions of the alcohol-soluble material of egg yolk. These were the crude sphingomyelin and the crude lecithin fractions.

In this experiment, pregnant guinea pigs were fed on: (*a*) regular diet; (*b*) regular diet pellets supplemented with the sphingomyelin fraction; (*c*) regular diet pellets supplemented with the lecithin fraction. In each case the fraction incorporated into the pellets was equivalent to the amount contained in a diet having 10 per cent whole egg yolk. When the offspring reached a weight of about 300 gm. they were challenged in the routine manner with the passive Arthus anaphylaxis. Measurements were made of the joint swelling and the rise in the blood serum level of the diphenylamine-reactive substance. These findings are presented in Table III.

TABLE III

Effect of a Crude Sphingomyelin (Alcohol-Insoluble) and a Crude Lecithin (Alcohol-Soluble) Fraction (from Egg Yolk) Added to Diets, Determined by Joint Swelling and Diphenylamine Reaction in Baby Guinea Pigs Challenged with Arthus Phenomenon

Diet	No. guinea pigs tested	DPA reaction*				No. guinea pigs Tested	Joint swelling			
		Δ log Io/I mean	± S.E.	*P*	Inhibition		M.S.I.	± S.E.	*P*	Inhibition
					per cent					*per cent*
Regular	12	0.048	0.007			17	2.41	0.23		
Alcohol-insoluble fraction	13	0.061	0.009	0.4–0.3	0	13	0.89	0.25	0.001	100
Alcohol-soluble fraction	13	0.017	0.015	0.1–0.05	65	13	1.3	0.23	0.01–0.001	99

* Difference in measured optical densities of serum before and after Arthus reaction. The Δ log Io/I equals Δ DPA.

It is seen in Table III that both fractions exerted a striking effect on the susceptibility to joint swelling. Nevertheless, a significant effect on the DPA level was not observed.

Experiment 4.—The fourth experiment was similar to the third except for the fractionation of the alcohol-soluble egg yolk material.

In this instance the egg lipids were dissolved in Skellysolve B. This was then put through 5 extractions with 85 per cent alcohol. Approximately 80 per cent of the material was extracted. This alcohol fraction was called fraction I. The 20 per cent of material remaining in Skellysolve B was called fraction II. These two fractions, equivalent to the fraction in 10 per cent whole egg yolk, were incorporated in guinea pig pellets. The results of the joint swelling and DPA determinations are given in Table IV.

It is seen in Table IV that with each fraction a significant inhibition of joint swelling and DPA reaction was observed.

TABLE IV

Effect of Experimental Diets (Skellysolve Fraction II and Alcohol Fraction I) on Arthus Phenomenon and DPA Reaction in Baby Guinea Pigs Born and Bred on Egg Yolk Fractions

Diet	No. guinea pigs tested	DPA reaction				No. guinea pigs tested	Joint swelling			
		Δ log *Io/I* mean	± S.E.	*P*	Inhibition		M.S.I.	± S.E.	*P*	Inhibition
					per cent					*per cent*
Regular	9	0.049	0.006			9	5.1	0.23		0
Skellysolve fraction II	9	0.019	0.005	0.01–0.001	61	10	1.6	0.11	0.001	90
Alcohol-soluble fraction I	8	0.012	0.006	0.01–0.001	76	8	1.5	0.13	0.001	90

Experiment 5.—In the preceding experiments the diets to be tested were given to the mother pigs during pregnancy. All animals tested were born and bred on the diets. Each experiment required approximately 5 months. To shorten this time, guinea pigs were purchased on the day of weaning; weanlings were then fed the experimental diets for periods of 3 to 5½ weeks and then challenged in the routine manner. The results are summarized in Table V *a*.

TABLE V *a*

Effect of Experimental Diets (Skellysolve Fraction II and Alcohol-Soluble Fraction I) on Arthus Phenomenon and DPA Reaction in Weanling Guinea Pigs Placed on Egg Yolk Fractions

Time	Diet	No. guinea pigs tested	DPA reaction				No. guinea pigs tested	Joint swelling			
			Δ log *Io/I*	± S.E.	*P*	Inhibition		M.S.I.	± S.E.	*P*	Inhibition
						per cent					*per cent*
3 weeks	Regular	5	0.047	0.010			5	5.4	0.41		
	Skellysolve fraction II	4	0.026	0.012	0.3	45	5	2.8	0.39	0.01–0.001	49
	Alcohol-soluble fraction I	5	0.018	0.005	0.1–0.05	62	5	2.3	0.28	0.01–0.001	58
5½ weeks	Regular	5	0.052	0.007			5	5.7	0.21		
	Supplemented with fraction I	3	0.011	0.003	0.05	79	3	1.6	0.37	0.01–0.001	72

This table shows that the alcohol (I) and Skellysolve (II) fractions gave a significant inhibition of the swelling index in the 3 weeks' experiment. The inhibition of the rise in DPA level observed was not statistically significant. Sufficient material of fraction I was available to run a few weanlings for 5½ weeks. It appeared that this longer period on the experimental diet augmented the inhibition of both the swelling index and also the rise in DPA level.

TABLE V *b*

Effect of Two Diet Supplements on Newborn and Weanling Guinea Pigs

Controls born and bred on stock diet			Experimental pigs born and bred on fraction II diet			Experimental pigs born and bred on fraction I diet			Weanling pigs on fraction II diet 2½ wks.			Weanling pigs on fraction I diet 2½ wks.		
Weight*	S.I.	Δ DPA	Weight	S.I.	Δ DPA	Weight	S.I.	Δ DPA	Weight	S.I.	Δ DPA	Weight	S.I.	Δ DPA
gm.			*gm.*			*gm.*			*gm.*			*gm.*		
280	6.5	0.046	330	1.9	0.008	260	2.1	0.012	320	2.5	0.001	280	3.2	0.020
305	5.5	0.031	270	1.9	0.018	220	1.3	0.023	290	3.5	0.048	240	1.7	0.039
260	4.8	0.087	265	2.3	0.049	280	2.0	0.030	320	2.5	0.008	240	2.6	0.010
360	5.0	0.043	390	1.3	—	240	1.4	0.013	270	2.9	0.048	220	2.7	0.016
410	5.1	0.065	340	1.0	0.036	310	1.6	0.018	300	2.5	—	230	1.5	0.006
340	4.2	0.051	320	1.8	0.013	280	1.3	0.002						
350	4.7	0.044	230	1.5	0.013	290	1.0	0.024						
440	4.6	0.033	290	1.4	−0.005	265	1.3	−0.024						
420	5.7	0.041	290	1.6	0.009									
			240	1.8	0.031									
Average...	5.1	0.049		1.6	0.019		1.5	0.012		2.8	0.026		2.3	0.018

* Individual guinea pig.
Controls (6 pigs), normal serum in ankle joint—S.I. = 1
" (" "), Δ DPA = 0

The complete data, obtained when the feeding of supplements during and following pregnancy was compared with the feeding for the 18 day period following weaning, are shown in Table V *b*. The former provided the greater protection.

Experiment 6.

An antibody solution of low titer was injected intracardially (1 ml. per kilo). This was followed 1 hour later by the injection of antigen (0.1 ml. of horse serum) into the left hind ankle joint. The accompanying swelling reaction and the rise in the DPA level 24 hours later was insignificant. On each successive day a different ankle joint was injected with the same amount of antigen. On the 5th day swelling measurements of the joint originally injected were again made and the DPA blood level was again determined. This injection of each joint, rotating clockwise, was repeated daily. Measurements of the left hind ankle were made again on days 9 and 14. During the latter part of this period all the injected joints became exceedingly tender and swollen. The measurements are shown in Table VI.

TABLE VI

Findings on Weanlings Born and Bred on Skellysolve Fraction II Diet Challenged with Arthus Reaction of a Different Ankle Joint Daily for 2 Weeks

	Day	No.	M.S.I. increase	Δ DPA rise mean
Stock diet plus fraction II	1	9	0.70	+0.006
	5	9	0.50	
	9	9	1.20	+0.015
	14	9	0.97	+0.038
Stock diet	1	10	1.1	+0.006
	5	10	1.4	+0.026
	9	10	2.2	+0.042
	14	7	3.0	+0.060

Table VI shows that although a weak antibody solution was used, both the DPA serum level and the M.S.I. of the joint originally inflamed rose progressively with the recurrent Arthus reactions in pigs fed on a stock diet. This progressive response to an antigen-antibody reaction persisted for 14 days after the injection of antibody. Subsequent injections of antigen had no significant effect on the joint swelling or DPA serum level. During the period of passive anaphylaxis, repeated doses of antigen induced inflammatory reactions of progressively greater intensity. As the duration of this period of daily Arthus reactions increased, the level of the DPA reactive substance in the blood rose steadily.

A group of guinea pigs similar to those raised on the stock diet was bred on the same diet supplemented with fraction II (Skellysolve B material). This group was challenged exactly as in Experiment 8. Whereas the control, stock diet pigs, developed chronically swollen, tender, painful ankle joints, the pigs on the test diet appeared refractory to challenges with antigen-antibody reactions. On palpation no gross changes were detected in their joints. The findings are given in Table VI.

Table VI shows that there was little or no physical or chemical evidence of inflammation in animals which were born and bred on the test diet. Furthermore histologic studies, made of the ankle joints of these two groups of pigs by Dr. Joseph D. Boggs, were essentially normal for the 8 pigs on the experimental diet. In contrast, each of the 6 pigs on the regular diet showed marked changes typical of chronic and subacute inflammation. The inflammatory reactions occurred both in the intrinsic structures and in the periarticular tissues, particularly in the latter.

Experiment 7.—In all these experiments a group of 6 guinea pigs were caged together on wire mesh. No attempt was made in any of these feeding experiments to determine how much of the pellet material was actually consumed by the group or how much by the individual animal. There was great wastage of food.

The following experiment was done to determine whether test material could be given intraperitoneally, and whether the administration of a diet supplement could be established on a quantitative basis.

Weanling guinea pigs were given intraperitoneally a daily dose of 1 ml. of a 1 per cent solution of crude sphingomyelin. This was estimated to be roughly comparable to what was consumed in the pellets administered in Experiment 3. The results are shown in Table VII.

It is seen in Table VII that there was an inhibition of both the joint swelling and of the rise in the DPA-reactive substance. Furthermore, the differences from the control animals were statistically significant. It appeared that this procedure was as effective as the feeding of crude sphingomyelin (Experiment 3).

TABLE VII

Intraperitoneal Feeding of 1 per Cent Crude Sphingomyelin Emulsion, 1 cc. Daily for 3½ Weeks

Experimental procedure	No. of animals tested	Δ log Io/I				No. of animals tested	Joint swelling			
		Mean	±S.E.	*P*	Inhibition		M.S.I.	±S.E.	*P*	Inhibition
					per cent					*per cent*
Control.....	5	0.052	0.007			5	5.7	0.21		
Sphingomyelin....	6	0.020	0.003	0.01–0.001	62	7	1.81	0.15	0.001	68

Experiment 8.—The purpose of this experiment was to determine whether the effects observed above would be obtained under carefully controlled feeding conditions.

Weanling guinea pigs (150 to 175 gm.) were placed in individual cages equipped with water bottles. In contrast to all animals reported above, none of these weanlings received any carrots or lettuce. Each animal was weighed just prior to and every 3 days during the course of the experiment. The diets were prepared by grinding guinea pig pellets and were placed in powdered state in cups wired so that only the head of the guinea pig could reach the food. The stock diet was made from guinea pig pellets (50 per cent Rockland and 50 per cent Dixie 22 per cent protein pellets) to which was added 0.1 per cent ascorbic acid. The test diet consisted of the stock supplemented with egg yolk powder in increasing concentrations according to the following schedule:—

Day	*Concentration of egg yolk, per cent*
1–3	4
4–6	8
7–11	15
12–16	20

In this study polyarthritis and monoarthritis were induced in pigs on the stock and the test diets. The same pooled antibody solution (anti-egg albumin) and the same antigen (crystalline egg albumin) were used throughout. Polyarthritis was induced by injecting 0.1 ml. of antigen, first in the left hind ankle joint and then daily in each of the other joints rotating clockwise. This was similar to Experiment 6 except that a stronger antibody solution was used.

Every 4 days a measurement was made of the left hind ankle joint. For animals on the stock diet, the DPA and M.S.I. values increased progressively. This began at once with animals which received the Arthus reaction and began on about day 6 with animals which received no antibody but only antigen daily in a joint. The changes were more marked than those seen in the guinea pigs with polyarthritis reported in Table VI. Animals with polyarthritis on the test diet failed to show after 5 days of challenges a progressive increase in DPA and M.S.I. values. They were sacrificed at this point for histological studies.

Guinea pigs with monoarthritis were not sacrificed until 11 days. These animals received a dose of antigen in the left hind ankle joint daily. On the stock and test diets there were four groups of pigs; *a* and *b* given antigen only, *c* and *d* challenged with the passive Arthus reaction. In the case of the animals on stock diet, group *b*, challenged with a daily Arthus reaction, the left ankle joints were markedly indurated. It became difficult to insert a hypodermic needle in the soft tissues, and accurate measurement of swelling was not possible. During this 10 day experiment, average measurements of weight gained and food intake were:—

Group *a* daily gain in weight, 6 gm.
" *b* " " " " , 0.9 gm. (severe arthritis)
" *c* " " " " , 6 gm., food intake 17 gm., supplemented 3.5 gm.
" *d* " " " " , 6 gm., food intake 17 gm. supplemented 3.4 gm.

Observations made on the four groups of pigs at the beginning and termination of this study are given in Table VIII.

TABLE VIII

Findings on Weanling Guinea Pigs Bred Individually on Weighed Diets and Challenged with Arthus Reactions of the Same Ankle Joint Daily

Diet	No. guinea pigs tested	Day of experiment	Joint change				DPA reaction			
			Swelling	±S.E.	*P*	Inhibition	Δ log Io/I mean	±S.E.	*P*	Inhibition
						per cent				*per cent*
Stock	Group *a* 8	1	0.14				−0.009			
		10	0.37				+0.104			
	Group *b* 11	1	0.40				+0.027			
		10	0.49							
Stock plus egg yolk	Group *c* 5	1	0.15				+0.028			
		10	0.19	0.06	0.1	50	+0.012	0.011	0.05	90
	Group *d* 5	1	0.39				+0.012			
		10	0.19	0.07	0.01	60	−0.003	0.003	0.001	100

It is seen in Table VIII that weanling guinea pigs on the test diet, groups *c* and *d*, failed to develop the progressive rise in joint swelling and DPA values noted in similar animals raised on a stock diet, groups *a* and *b*. This inhibition was significant for the passive Arthus reaction. In brief, the results obtained

under carefully controlled feeding conditions were similar to those reported above in colonies of guinea pigs which consumed unmeasured amounts of test diets.

DISCUSSION

The observations presented in this report are believed to indicate that under certain conditions, some lipid substance of egg yolk will inhibit an anaphylactic reaction in the young guinea pig. A single dose of the material given prior to the challenge with the passive Arthus phenomenon had no effect. Likewise, the material had little effect if fed daily to or injected intraperitoneally into adult pigs. In contrast, guinea pigs born and bred on a stock diet supplemented with the material received a high degree of protection against anaphylactic arthritis. A significant degree of protection was also obtained in weanling pigs (150 to 175 gm.) which received the supplemented diet for 2 to 4 weeks prior to being challenged. The experiments show that the diet supplement was effective when: (*a*) administered in pellets to guinea pig colonies; (*b*) injected daily in known quantities intraperitoneally; (*c*) supplied quantitatively in powdered form to the animal bred in an individual cage.

Attention is called to the possibility that these experimental findings on mono- and polyarthritis may be pertinent to the genesis of the rheumatic state. The factors present among "less privileged" persons, which favor the development of rheumatic fever, are not well defined. There is, nevertheless circumstantial evidence that lipids may play a role. For example, clinicians and pathologists have long observed that rheumatic fever occurs rarely in association with diseases characterized by lipemia (*e.g.*, diabetes mellitus, myxoedema, and nephrosis); whereas it occurs commonly in association with Graves' disease in which there is a low level of blood lipids. Furthermore, it has been shown by Stollerman *et al.* that in patients who develop rheumatic fever there is a low serum level of streptolysin S inhibitor, which appears to be a phospholipoprotein complex (6, 7). Moreover, several nutritional studies have indicated that there is a low intake of eggs among children who develop rheumatic fever, and that supplementary feedings with large amounts of whole egg yolk powder may prevent rheumatic recurrences (8–10). Recent reports have indicated that there may be a relation between a low blood level of serum phospholipids in rheumatic fever and the consumption of eggs (11, 12). Whether rheumatic fever is an anaphylactic reaction and whether the consumption of egg yolk in childhood is a factor in its pathogenesis remain to be determined.

SUMMARY

Whole egg yolk incorporated as a supplement in the diet of baby guinea pigs afforded protection against anaphylactic arthritis as determined: (*a*) by measurement of joint swelling; (*b*) by the rise in serum level of some substance which reacts with diphenylamine; (*c*) by histologic examination.

The active material in egg yolk was shown to be in the alcohol-soluble fraction. Attempts to identify the active material with any known lipid have to date been unsuccessful.

In the screening of lipid substances for protection against anaphylactic arthritis, it is shown that the weanling guinea pig is suitable, that a 3 week period is adequate, and that the test substance can be administered satisfactorily in the diet.

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